Symposium 20: Single Molecules Meet Systems Biology

3919-Symp
Single Molecules in Single Cells: A System-Wide Quantification of Gene Expression
Sunney Xie.
Harvard University, Cambridge, MA, USA.
Our group studied gene expression in living bacterial cells with single-molecule sensitivity with millisecond time resolution and nm spatial precision. We reported the first movie of protein production, one molecule at a time, binding and unbinding kinetics of transcription factors on DNA, and their single-molecule events that change the cell's phenotype. We recently conducted system-wide studies of transcriptome and proteome with single-molecule sensitivity in a single cell. We are investigating the phenomenon of persisters, abnormal and rare bacterial cells that have the same genes as normal cells, but are phenotypically drug tolerant, hoping to provide clues for developing tuberculosis drugs.

3920-Symp
Probing Intracellular Kinetics at the Level of Single Molecules
Johan Elf.
Uppsala University, Uppsala, Sweden.
I will present our resent advancements in tracking individual freely diffusing fluorescent proteins molecules at high time resolution in the cytoplasm of bacterial cells. *In vivo* tracking of individual proteins molecules makes it possible to study kinetics high time resolution without synchronizing the population of molecules. For example by monitoring the kinetics of the response mediator RelA we have developed a single molecule assay to study stress response and starvation at the level of individual bacteria.
The RelA protein binds to a small fraction of ribosomes, where it synthesizes the global transcriptional regulator ppGpp in response to amino acids deprivation. This the ppGpp molecule binds to the RNAP and rapidly reprograms the cell for the new environment, in what is called the stringent response. While *E. coli* contains on average about 100 RelA molecules, using a photo-activatable fluorescent probe we can activate only a few fluorescent molecules per cell at any given time and track them at high time resolution. The procedure can be repeated many times to get accurate statistics for in individual cells.
When the cell grows exponentially, RelA trajectories closely resemble trajectories of fluorescently tagged ribosomal proteins (D~0.4um$\perp$2/sec as compared to D~0.3 um$\perp$2/sec for ribosomes). After nutritional downshift, RelA binding kinetics changes rapidly and the protein diffuses very fast (D~3.5um$\perp$2/sec) as if it only binds to ribosomes transiently. The assay has made it possible to study the rapid and transient stringent response in individual cell as well as the heterogeneity in the stress response over the population.

3921-Symp
Error Minization in Lateral Inhibition Circuits
Naama Barkai.
Weizmann Inst, Rehovot, Israel.
No Abstract.

3922-Symp
Signaling Dynamics at the Single Cell Level
Michael Elowitz.
California Inst Tech, Pasadena, CA, USA.
Our lab studies the dynamics of gene circuits at the single-cell level. This talk will focus on signal encoding schemes used in different prokaryotic and eukaryotic signal transduction systems. In particular, I will discuss new, dynamic encoding schemes and their possible functions.

Symposium 21: Mechanism of Electromechanical Coupling in Voltage-gated Ion Channels

3923-Symp
The Structural Basis of Voltage Sensing
Roderick MacKinnon.
Rockefeller Univ, HHMI, New York, NY, USA.
No Abstract.

3924-Symp
The Structural Basis of Gating Currents: Insights from Fluorescence Spectroscopy
Francisco Bezanilla.
Univ Chicago, Chicago, IL, USA.
The S4-based voltage sensor found in voltage-gated ion channels and in voltage dependent phosphatases plays a fundamental role in controlling cellular excitability. Although recently the combination of electrophysiology, mutagenesis, spectroscopy and X-ray crystallography have delineated the basic molecular correlates of the voltage sensor operation, we still are far from understanding the physical basis of the sensing (gating) currents. The study of sensing currents show that the sensor visits three main states: Resting, Active and Relaxed (inactivated). Furthermore, between the Resting and Activestatesthere is at least another major state that can be modulated by mutations. In addition, we know with a fair degree of precision, the kinetics and magnitude of the charge moving among the different states. However, the structural correlates of these states and transitions are for the most part unknown. Fluorescence spectroscopy , concomitant with electrophysiology, has been used to detect conformational changes. Thus, local changes within the sensor are detected by following modifications in the environment of a fluorophore probe situated in specified sites and changes in conformations have been detected and quantified as intramolecular distance changes using the LRET technique. These techniques are especially critical in characterizing the short-lived Active state that is populated by positive potentials and spontaneously decays to the Relaxed state within tens to hundreds of milliseconds. As the Relaxed state is stable, it is most likely the state that X-ray crystallography has caught. Fluorescence spectroscopy confirmed that indeed there are three main states and that they each correspond to different conformations of the voltage sensor. In addition, it has provided the kinetics of the conformational changes and estimates of the relative movements of the voltage sensor while evolving from Resting to Active to Relaxed. Support: NIHGM030376.

3925-Symp
Dynamics of Voltage-Sensor Movement in Sodium Channels
Todd Scheuer.
Univ Washington, Seattle, WA, USA.
Voltage-gating of ion channels is driven by the S4 segment residing in a gating pore in proximity to S1, S2 and S3 segments of the voltage sensing module of the channels. Molecular modeling using Rosetta has proposed detailed movements of the S4 voltage sensor in the gating pore during gating. As it transits between deactivated and activated conformations, voltage sensor arginines are hypothesized to make sequential ion pairs with negatively-charged residues in surrounding transmembrane segments that catalyze the transmembrane movement of the voltage-sensor positive charges; the charge-charge pairs also prevent movement of solution ions through the gating pore. We have used two approaches to test this model. First, using mammalian sodium channels we have shown that substitutions of glutamine for the two most extracellular arginines allows current through the gating pore at hyperpolarized potentials where predicted charge-charge interactions are missing due to mutation, but not at depolarized potentials where the remaining more intracellular and unmutated arginines are predicted to form pairs and block the gating pore. Conversely, glutamine substitutions for arginines 2 and 3 allows gating pore current at depolarized but not hyperpolarized potentials. In a second approach, we have used bacterial NaChBac sodium channels and substituted pairs of cysteines for negatively-charged amino acids in S2 and positively-charged arginines in S4 that are predicted to interact during gating. We demonstrate rapid disulfide bond formation between the substituted cysteines that is voltage (and conformation) dependent. The time and voltage-dependence of these disulfide interactions provide experimental evidence for sequential formation of specific ion pairs as the voltage sensor moves within the gating pore during channel activation. The combination of these experimental approaches gives an increasingly detailed picture of the conformations and interactions of S4 in the gating pore during voltage-sensor activation.

3926-Symp
Molecular Movements within the Voltage-Sensor Domain of a Potassium Channel
Fredrik Elinder.
Linköping Univ, Linköping, Sweden.
Voltage-gated ion channels play critical roles in all biological systems, for instance in transmitting the nervous impulse and initiating the heart beat. Changes in the membrane potential are detected by a voltage sensor in the channel, leading to opening and closing of the ion-conducting pore. While the atomic structure is known of a voltage-gated K channel in an activated state, the chain of molecular events leading to channel gating is known in less detail. We have used disulfide-linking techniques in combination with electrophysiology to probe molecular motion within the channel protein. Two amino-acid residues are mutated to cysteins and if these residues are close enough to each other it is possible to make and break disulfide or cadmium bridges, detected as a change in channel function. The analysis also gives information about if